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WADC TECHNICAL REPORT 54-345

THE TOXICITY OF CERTAIN AROMATIC PHOSPHATE ESTERS

JOSEPH F. TREON
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UNIVERSITY OF CINCINNATI

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WRIGHT AIR DEVELOPMENT CENTER

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WRIGHT AIR DEVELOPMENT CENTER
AIR RESEARCH AND DEVELOPMENT COMMAND
UNITED STATES AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

Carpenter Litho & Prtg. Co., Springfield,O.
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FOREWORD

The investigation reported herein was performed in the Kettering Laboratory in the Department of Preventive Medicine and Industrial Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio, under Contract No. 33(038)26456 with the Aero Medical Laboratory, Directorate of Research, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio.

The work was carried out under RDO No. 698-31,"The Toxicity of Developmental Air Force Materials" with George Kitzes, Ph. D., as contract monitor.

Actively engaged in the project were Joseph F. Treon, Ph. D., Frank P. Cleveland, M. D., John Cappel, B. S., Robert Boller, M. S., Frederic E. Shaffer, M. S., Leo Hartmen, B. S., Irving Wittow, B. S., John Torbeck, B. S., William Wagner, B. S., Jose Gotay, B. S., Thomas Gahegan, James Finan, James Boyd, James Coomer, Donald Brown and James Gosney.

The work was done and the report prepared under the general supervision of Francis F. Heyroth, M. D., Director of the Division of Industrial Toxicology in the Department of Preventive Medicine and Industrial Health, College of Medicine, University of Cincinnati. The report was edited and approved by Robert A. Kehoe, M. D., Director of the Department.

The experiments reported herein were performed according to the "Rules regarding animal care" as approved and adopted by the American Medical Association.

ABSTRACT

Although each of seven commercial grades of tricresyl phosphate (when administered to rabbits in one dose by stomach tube) was found to be moderately toxic, no one of them was as toxic as pure triorthocresyl phosphate. Rats were quite resistant to the toxic effects of these substances. Two samples of xylenol phosphate and two formulations containing 5%, or less, of triorthocresyl phosphate were less toxic than were the commercial grades of tricresyl, when given by stomach tube to animals.

The commercial tricresyl phosphates readily penetrate the intact or abraded skin of rabbits, giving rise to lethal results when sufficient quantities are applied thereon. The xylenol phosphates and the two formulations were also absorbed through the skin, but larger quantities of these materials, as compared with the commercial grades of tricresyl phosphate, had to be applied upon the skin to kill rabbits.

All of these substances yielded cumulative effects when they were applied upon the skin of rabbits in spaced multiple doses.

Two of the commercial samples, F and H, appeared to be more toxic than the others, whether they were administered in one dose by stomach tube, or were applied in a series of doses upon the skin.

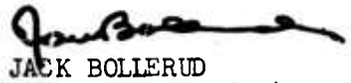
The saturation of air with the vapor of commercial tricresyl phosphates at ordinary temperatures (25°C) is not likely to result in hazard, since the vapor pressure of these compounds is quite low. Respiratory exposure to a mist or spray of these materials, on the other hand, may well be highly hazardous under suitable conditions. Toxic concentrations of vapor may be reached when the compounds are subjected to elevated temperatures. The compounds decompose at temperatures in excess of 566°C and under such conditions the toxicity of the resultant mixture of materials in the atmosphere depends, largely but not entirely, on the extent to which the original tricresyl phosphate has been decomposed. The toxicity of the thermal decomposition products, themselves, within the range of temperature explored in these experiments, is of about the same order as that resulting from the decomposition of a typical paraffinic lubricating oil at the same temperature.

Precautions should be taken to prevent prolonged contact of the commercial tricresyl phosphates with the skin, and if such contact occurs, the skin should be flushed generously with water.

PUBLICATION REVIEW

This report has been reviewed and is approved.

FOR THE COMMANDER:



JACK BOLLERUD
Colonel, USAF (MC)
Chief, Aero Medical Laboratory
Directorate of Research

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INTRODUCTION

The experiments detailed in this report were performed for several purposes. These include:

1. Determination of the immediate toxicity of seven samples of commercial tricresyl phosphate, a sample of triorthocresyl phosphate, two samples of xylenol phosphate, and two formulations, MLO-6405 and MLF-6400 (containing triorthocresyl phosphate), when given orally to rabbits and rats and when maintained upon the intact or abraded skin of rabbits for 24 hours;

2. Testing the cumulative action of a series of doses of the seven samples of commercial tricresyl phosphates and the two formulations, when maintained in contact with the skin of rabbits for two hours on each of five days per week over a period of ten weeks;

3. Observing the response of animals of several species to the inhalation of the vapors and the decomposition products of tricresyl phosphate (Monsanto) formed by dropping this compound upon a heated Inconel surface;

4. Describing the histopathologic changes encountered in the tissues of animals exposed to these materials under various experimental conditions.

The tests of the vapor and the fog of tricresyl phosphate were conducted for Monsanto Chemical Company, which has permitted the incorporation of the data in this report.

PROPERTIES OF MATERIALS

Sample A had a very high content of triorthocresyl phosphate, since it had been prepared from a sample of cresylic acid shown by analysis to contain 97.5 per cent of orthocresol. The molecular weight of triorthocresyl phosphate is 368.4.

Seven samples (B to H) were regular commercial grades of the mixed esters of tricresyl phosphates, as supplied by three manufacturers.

Samples B, D, E and F were derived from cresylic acids obtained from petroleum, whereas samples C, G and H were derived from cresylic acid obtained from coal tar.

Sample D was said to have been prepared from a cresylic acid which contained the following ingredients:

Orthocresol	3.0 per cent (maximum)
Metacresol	26.7 per cent
Paracresol	31.7 per cent
Xylenols	38.3 per cent
Phenols and higher Phenols	1.0 per cent

Sample E was reported to have been prepared from cresylic acid containing less than 0.5 per cent of the ortho isomer, whereas sample G was said to have been made from cresols containing about 1.5 per cent of the ortho isomer.

Samples F and H were said to contain higher concentrations of the ortho isomer than the other 5 commercial grades of tricresyl phosphate. Sample F was reported to have been prepared from cresylic acid containing at least 5 per cent of the ortho isomer, whereas the cresols used in preparing sample H contained from 3 to 10 per cent of the ortho isomer.

From statistical calculations made on the assumption that the various isomeric cresols undergo phosphorylation at the same rate, a sample consisting of 3 per cent of orthocresol and 48.5 per cent of each of the meta and para isomers would give rise to the formation of tricresyl phosphate containing the various possible esters in the following proportions (letter of July 11, 1952, from E. B. Fisher, Mondanto Chemical Company to Mrs. I. R. Campbell, The Kettering Laboratory):

Triortho cresyl phosphate	0.0027	per cent
Tripara cresyl phosphate	11.41	per cent
Trimeta cresyl phosphate	11.41	per cent
Diortho, monometa cresyl phosphate	0.14	per cent
Diortho, monopara cresyl phosphate	0.14	per cent
Monoortho, dimeta cresyl phosphate	2.12	per cent
Monoortho, dipara cresyl phosphate	2.12	per cent
Dipara, monometa cresyl phosphate	34.22	per cent
Dimeta, monopara cresyl phosphate	34.22	per cent
Monoortho, monometa, monopara cresyl phosphate	4.23	per cent

A commercial sample of tricresyl phosphate was supplied by Monsanto Chemical Company for the work sponsored by them concerning the toxicity of the vapor and its pyrolytic products.

In addition, two samples of xylenol phosphates, $O = P(O\text{-}C_6H_3(CH_3)_2)_3$, were tested. One, sample I, produced from cresylic acid from coal tar, contained approximately 22 per cent of meta and para cresols, less than 2 per cent of orthocresyl, the remainder consisting of xylenols and higher alkyl phenols. The other, sample J, prepared from cresylic acid from petroleum, contained approximately 20.5 per cent of meta and para cresols and less than 2 per cent of orthocresols, along with some xylenols and higher alkyl phenols. The molecular weight of xylenol phosphate is 410.4.

Ten of these samples were analyzed for their content of the ortho-tolyl group on a cresylic acid basis by Shell Development Company, Emeryville, California, by means of its absorption in the infrared region of the spectrum. The values obtained were as follows:

Sample	Per cent O-tolyl groups (on a cresylic acid basis)
A	100.0
H	19.2
F	15.5
G	4.0
D	4.0
E	2.3
B	1.9
C	1.3
J	1.3
I	0.3

Sample MLF-6400 was a clear, colorless aviation gasoline, containing 5 ml of triorthocresyl phosphate per gallon.

Sample ML0-6405 was a yellow fluid of the following composition:

Di-2-ethylhexyl sebacate	90.6 per cent
Triorthocresyl phosphate	5.0 per cent
Acryloid HF-890 or HF-25	3.9 per cent
Phenothiazine	0.5 per cent
Silicone antifoam (probably DC-4)	0.001 per cent

EXPERIMENTAL METHODS

Oral Administration of a Single Dose.

A measured amount of one of the desired materials was introduced into the stomach of each of a series of healthy female rabbits from an hypodermic syringe by means of a rubber catheter (F20) passed through the esophagus. To make certain that the entire dose entered the stomach, the tube was flushed with 4 or 5 ml of water. Comparable dosages of the materials were administered to rats; a measured amount of the undiluted material, contained in a syringe, being expelled through a blunt, 6cm, 17-gauge hypodermic needle, which had been filled completely with the material before its insertion into the esophagus. Since by this procedure the volume of material was measured by displacement from the syringe, it was not necessary to flush out the syringe with water. The animals were weighed daily until any losses sustained following the administration of the material had been regained, after which they were weighed once per week until they were killed.

Five of the substances (samples A, C, D. I, and J) were also given to rabbits as a 5 or 10 per cent (V/V) solution in peanut oil.

Application Upon the Skin of Rabbits.

By means of the technique of Draize, Woodard and Calvery, the desired amount of the undiluted material was maintained for 24 hours in contact with the intact skin of each of 3 female rabbits and with the abraded skin of each of 3 others. If fatalities resulted in either group, a corresponding experiment was carried out at a lower dosage. The dosage was decreased until

it was tolerated by all of the members of the group. Pursuant to this method, the hair was clipped from an area of skin 6 to 7 inches wide and completely encircling the trunk of each rabbit. The entire trunk was covered with a sleeve of dental dam which fitted tightly at the cephalad and caudad edges of the clipped area and loosely over the intervening area. During the 24-hour period of contact the rabbit was kept in stocks (Figure 1). The outermost layer of the skin of certain rabbits was abraded by scratching thin furrows, 7.5 cm in length and a few millimeters apart, with an hypodermic needle, prior to the application of the material to be tested. At the end of 24 hours, the material was removed from the skin of the animals by washing with water and a sulfonated oil, "pH6".

Repetitive Applications Upon the Skin of Rabbits.

In a second series of experiments, groups of 3 female rabbits were kept in stocks in the supine position (Figure 2), the hair having been clipped previously from the abdominal skin. A dosage of 5.0, 2.0, 1.0, 0.50 or 0.25 ml of one of the 7 commercial grades of tricresyl phosphates or of one of the formulations (ML0-6405, MLF-6400) was maintained in contact with the intact abdominal skin for 2 hours on each of 5 days per week over a period of several weeks. The material was removed each day by washing in the manner described above.

Volatilization Without Decomposition.

For the volatilization of tricresyl phosphate, two flat-bottomed cylindrical bubbling towers (2.94" x 13") in parallel (Figure 3) were employed, a fritted glass thimble (0.94" x 3.75") being immersed in the liquid contained in each. These towers were kept in a bath of Nujol maintained at 109°C. Air passed through the two bubbling towers at rates of 5.0 and 6.3 liters per minute, respectively, as measured by a rotameter in each line, the streams merging in a glass tube leading to a manifold immersed in a cooling bath before entering the chamber.

At the start of the experiment 550 milliliters of tricresyl phosphate were placed in each of the two bubbling towers. It was found that the concentration of vapor within the chamber became less on the second day of exposure and still less on the third. It is

thought that the tricresyl phosphate might have hydrolyzed somewhat while standing overnight, possibly because it had absorbed moisture from the air that passed through it during the day. In order to maintain the atmospheric concentrations as uniform as possible, the material remaining in the towers was replaced by a fresh supply on the fourth day of the experiment. Had sufficient material been on hand, this would have been done daily in order to attain a somewhat higher average concentration throughout an experiment.

The animals were confined in a 223-liter cylindrical steel chamber (Figure 4) coated on the inside surface with a baked plastic. The behavior of the animals could be observed through a sheet of clear pliable plastic membrane, which sealed the otherwise open front end of the chamber. An interposed heavy wire-mesh screen prevented the animals from breaking the plastic membrane. The contaminated air entered the chamber near the top of the rear wall, on which was mounted a circulating fan. Effluent air was withdrawn through an outlet located toward the front of the lower part of the chamber, by maintaining a slight negative pressure.

Generation of a Fog from a Heated Inconel Surface.

A rotameter for measuring the flow of air, a furnace for volatilizing and decomposing the liquid, a semi-potentiometer (see below), a cooling manifold, and the chamber(223-liters) for confining the animals during their exposure, are shown in Figure 4. In these experiments the smaller rotameter, to the right in the photograph, was not employed, and all air (20 liters per minute) that entered the chamber was passed through the furnace. The cooling coils were packed in the bath shown and surrounded by crushed ice.

The body of the furnace consisted of a 26-inch length of cold-drawn seamless Inconel tubing, having an outside diameter of 1.5 inches and a wall-thickness of 0.049 inch. Tricresyl phosphate was dropped upon the inner surface of the tube from a burette through an Inconel tube welded to the upper surface of the heated tube near its midpoint. This side tube had a length of 6 inches, an outside diameter of 0.675 inch,

and a wall-thickness of 0.091 inch. Another piece of Inconel tubing, 3 inches in length and having an outside diameter of 0.438 inch and a wall-thickness of 0.049 inch, was also welded to the upper portion of the furnace tube, to permit the insertion of a thermocouple in such a manner as to measure the temperature at the upper interior surface of the furnace. The distance from center to center of the two smaller tubes, which were parallel to one another and perpendicular to the furnace proper, was 1.75 inches.

The outside of the furnace was wrapped with 2 layers of molding mica. The middle 10-inch portion of the length of the furnace was electrically heated by means of 20 feet (9 wrappings to the inch) of A. W. G. Number 20 Tophet "C" wire, which has a resistance of 0.659 ohm per foot at 68°F. The wire was insulated with approximately 0.75 inch of diatomaceous earth and wrapped on the outside with asbestos cloth.

The temperature of the wall of the furnace was measured by an iron constantan thermocouple attached to a semi-potentiometer calibrated against a student potentiometer. The thermocouple was in contact with the inner upper wall of the furnace about 1 inch downstream from the point at which the droplets of material fell upon its lower interior surface.

After the furnace had been brought to the desired temperature of either 1050°F., or 1250°F., the hydraulic fluid was dropped from a burette at the rate of 0.067 to 0.245 ml per minute (cf Table 2), 0.100 to 0.167 ml per minute being the usual rate.

SAMPLING AND ANALYSIS OF CONTAMINATED AIR

While the undecomposed vapor of tricresyl phosphate was being metered into the air in the chamber, 4 samples of air were taken from the chamber each day, by drawing air therefrom, at the rate of 3.4 liters per minute, through 2 absorption towers (2.25" x 13" outside diameter), each containing 100 ml of redistilled ethyl alcohol. The sample passed through a coarse fritted-glass disc (1.13" in diameter) immersed in the solvent and set parallel to the bottom of the tower and about 0.2" above it. The

content of tricresyl phosphate so collected was determined by measuring the transmission of ultraviolet radiations of wave length 264 m μ, by means of a Beckman spectrophotometer.

The alcohol used to extract the vapor of tricresyl phosphate from the atmosphere was freed from ultraviolet-absorbing impurities by the following procedure:

About 1750 ml of 95 per cent ethyl alcohol were permitted to stand overnight with 10 g of pellets of KOH and 7 g of aluminum foil (0.003"). The mixture was then refluxed for half an hour and distilled at 78.5°C., the first 20 ml being rejected.

In experiments involving the exposure of animals to the products formed by the thermal decomposition of tricresyl phosphate, over periods of time no longer than 7 hours, from 3 to 5 samples of air were collected from the chamber for analysis, depending upon the length of the period of exposure. When the exposure was repeated on a subsequent day, a corresponding number of samples was collected. Analyses were made for the following materials: Tricresyl phosphate, free cresols, and carbon monoxide.

The cresols were determined colorimetrically after coupling with diazotized sulfanilic acid, by methods described by Warshowsky and Schantz for the meta isomer, and by Schmidt for the para isomer. Samples of air for the determination of either tricresyl phosphate of free cresols (meta and para isomers) were passed at the rate of 6.8 liters per minute for 30 to 45 minutes through a fritted-glass disc scrubber and an impinger, in series. Each tower contained 100 ml of a 1 per cent sodium hydroxide solution. In analyzing for the free cresols, 1 ml of a 20 per cent solution of sodium carbonate was added to an aliquot of 6 ml or less, in a 10 ml glass-stoppered graduated cylinder. After the solution had been diluted to 8 ml, 2 ml of diazotized sulfanilic acid solution were added. The latter was prepared by adding 0.75 ml of a 5 per cent solution of sodium nitrite to 0.75 ml of a solution of sulfanilic acid contained in a 25 ml volumetric flask previously cooled in an ice-bath. The sulfanilic acid solution was prepared by dissolving 4.5 g of the acid in 45 ml of concentrated hydrochloric acid and diluting it to 500 ml with water. After the partially diazotized solution had been cooled for 5 minutes, an additional increment of 3 ml of sodium nitrite solution was added, the mixture was further cooled for 15 minutes and then diluted to 25 ml. The solution was prepared daily and kept cold.

Curves relating the transmission of light of wave-lengths 425 mμ and 500 mμ to the concentration of meta cresol over the range of 3 to 49 micrograms per 10 ml are shown in Figure 5. In making the necessary measurements, the comparison cell contained a solution prepared similarly except for the omission of cresol. Corresponding curves for para cresol over the range of 10 to 120 micrograms per 10 ml are shown in Figure 6. By the use of the following simultaneous equations, the amounts of meta and para cresol could be calculated.

For the determination of metal cresol (γ):

p-cresol + 4.31 m-cresol = apparent p-cresol (425 mμ)
p-cresol + 1.13 m-cresol = apparent p-cresol (500 mμ)

3.18 m-cresol = difference in apparent p-cresol at the 2 wave-lengths

$$(\gamma)\ \text{m-cresol} = \frac{\text{difference in apparent p-cresol}}{3.18}$$

For the determination of para cresol (γ):

$$\text{m-cresol} + \frac{1}{4.31}\ \text{p-cresol} = \text{apparent m-cresol (425 m}\mu\text{)}$$

$$\text{m-cresol} + \frac{1}{1.13}\ \text{p-cresol} = \text{apparent m-cresol (500 m}\mu\text{)}$$

$$(\gamma)\ \text{p-cresol} = \frac{\text{difference in apparent m-cresol}}{0.653}$$

The validity of this method for the simultaneous determination of the two isomers was demonstrated by the following determinations on samples containing both of them in known amounts. Analysis of a sample prepared to contain 12.30 γ of m-cresol and 9.962 γ of p-cresol yielded 13.50 γ and 9.95 γ, respectively. A second sample prepared to contain 24.60 γ of m-cresol and 9.96 γ of the para isomer yielded 25.90 γ and 10.7 γ, respectively.

The values designated in Table 23 as free cresols represent the sums of the two isomers. From 60 to 70 per cent of the total amount of free cresols present in the chamber, as the result of decomposition at 1050°F.,

was found to consist of the meta isomer, but when the temperature of the furnace was 1250° F., only the meta isomer was present. Since a sample of tricresyl phosphate, analyzed after hydrolysis, contained approximately 65 per cent of the meta, and 35 per cent of the para isomer, it appears likely that the latter underwent decomposition to unidentified fragments at the higher temperature.

For the determination of undecomposed tricresyl phosphate present in the chamber, a 5 ml aliquot portion of the sample, collected in the manner indicated for the determination of cresols, was hydrolyzed by refluxing it for an hour with 10 ml of 45 per cent aqueous sodium hydroxide; it was then acidified with concentrated hydrochloric acid and distilled until 300 ml had been collected. The usual cresol method was then applied to the distillate.

Carbon monoxide was determined by a modification of the method of Bland. The samples were collected by passing air at the rate of 4.7 liters per minute for 5 minutes through the absorption train shown in Figure 7, by means of a Gast Rotary Blast and Suction Pump located beneath the table and not shown in the photograph. The first U-tube contained glass beads and a 10 per cent aqueous solution of lead acetate, while the second U-tube, which contained sulfuric acid (S.G. 1.84) and glass beads, was surmounted by a long, narrow tube packed from top to bottom with successive layers of silica gel (impregnated with a cobalt salt to indicate the presence of moisture), magnesium perchlorate (Anhydrone), a sodium hydrate asbestos absorbent (Ascarite) and a second layer of magnesium perchlorate. Air issuing from the top of this tube passed through a tube containing iodine pentoxide heated to 115°Centigrade. Iodine liberated by the reaction

$$I_2O_5 + 5CO \longrightarrow 5CO_2 + I_2$$

was collected in a bubbler containing 5 ml of a 3 per cent aqueous solution of potassium iodide. The rate of flow was measured by an upright manometer shown in Figure 7. The contents of the bubbler were titrated with 0.001 N arsenious acid, employing starch as indicator,

$$H_3AsO_3 + I_2 + H_2O \longrightarrow H_3AsO_4 + 2HI$$

One milliliter of 0.001 N arsenious acid is equivalent to 0.070025 mg of carbon monoxide. In calculating the results, allowance was made for the previously determined traces of free iodine in the volume of the solution

of potassium iodide present in the bubbler.

Each of the following concentrations of the various contaminants of the air of the chamber, in ppm by volume in air at 84°F. and 745 mm of mercury, is equivalent to the concentration of 1 mg per liter.

Compound	1 mg/l ⟷ ppm
Tricresyl phosphate	68.6
Cresol	233.8
Carbon monoxide	902.7

EXPERIMENTAL RESULTS

The Immediate Toxicity of the Materials When Administered Orally.

The data relating to the oral administration of each of the undiluted samples A to H to rabbits and rats are given in Tables 1 and 3, respectively. Comparable data on five of these samples (A, C, D, I and J), when given in solution in peanut oil to rabbits, are presented in Table 2. The minimum lethal dosages have been summarized in Table 4.

When given without a diluent to rabbits, the lethal dose of Sample A was too small for accurate measurement (Table 1). When this material was given in solution in peanut oil, each of 3 rabbits tolerated the dosage of 55 mg per kg of body weight, but the dosage of 80 mg per kg was lethal to 1 of 3 rabbits, and larger dosages resulted in an higher incidence of mortality (Table 2). Rats were less susceptible to sample A, since all of 4 survived, after having been given the undiluted dosage of 3.2 g per kg of body weight (Table 3), but larger dosages resulted in the death of some among the groups given this material. Sample A, which consisted chiefly of the ortho isomer of tricresyl phosphate, was more toxic for rabbits or rats than was any one of the mixed commercial grades of tricresyl phosphate (Table 4).

The seven commercial samples of tricresyl phosphates (samples B to H inclusive) appeared to fall into two classes, with respect to their toxicity for rabbits,

samples F and H, which were reported to have a higher content of the ortho isomer than the others, being the more toxic (Tables 1 and 4). The minimum lethal dosage of sample F was greater than 0.12 and less than 0.18 ml per kg, and that of sample H was between 0.18 and 0.28 ml per kilogram. Samples C, D, G and E did not differ significantly among themselves in toxicity, the minimum lethal dose of each being greater than 0.42 and less than 0.62 ml per kg; sample B was slightly less toxic, its value lying between 0.62 and 0.94 per kg (Tables 1 and 4.

Rats were more resistant to the seven commercial grades since only an occasional rat died when the dosage of 10.7 ml per kg was given, and many survived after larger dosages had been given (Tables 3 and 4).

When given undiluted to rabbits, the xylenol phosphates (samples J and I) were less toxic than any of the seven samples (B to H inclusive) of commercial tricresyl phosphates (Tables 1 and 4). The dosage of 2.1 g of sample I per kg was tolerated by 3 rabbits, but larger dosages resulted in some fatalities. Sample J was even less toxic, since among 6 groups, each consisting of 4 rabbits, to which were given, respectively, the dosages of 16.0, 10.7, 7.1, 4.7, 3.2 and 2.1 ml per kg, only 1 rabbit given the dosage of 16.0 ml per kg and 1 given that of 4.7 ml per kg died (Table 1). Rats were also resistant to samples I and J (Tables 3 and 4).

When sample C was given in peanut oil rabbits were more susceptible than when no diluent was used, but dilution had no effect upon the toxicity of sample D. The potentiating effect of the oily diluent was most evident in the case of rabbits given the xylenol phosphates (samples I and J). The minimum lethal dose of sample I, when given in solution in peanut oil to rabbits, was greater than 0.28 and less than 0.42 ml per kg (Tables 2 and 4), whereas the corresponding value for sample J was between 1.4 and 2.1 ml per kilogram.

The occasional variations in the dosage-mortality relationship encountered in the experiments with these materials may be ascribed to the diarrhea that occurred following their administration thereby shortening the period of their absorption from the alimentary tract. Signs attributable to damage of the central nervous system were also observed.

The oral administration of a lethal dosage of samples A to J to rabbits and rats resulted in non-specific degenerative changes in the brain, liver, and kidneys. Hyperemia was observed in other viscera as well, following the administration of sample A (tri-o-cresyl phosphate) or of either of the samples of the xylenol phosphates (I and J). Rabbits that survived larger dosages of samples C, F, G, or J and rats that survived larger dosages of A, C, or F had diffuse degenerative changes in only the liver and kidneys.

The fate of rabbits and rats given a single oral dose of either sample ML0-6405 or MLF-6400 is shown in Table 5. The minimum lethal dose of ML0-6405 for rabbits was greater than 1.4 and less than 2.1 ml per kilogram. The corresponding value for rats lies between 7.1 and 10.7 ml per kg (Table 5): Aviation gasoline containing TCP (MLF-6400), when administered correspondingly to animals, was much less toxic than ML0-6405, its minimum lethal dose for rabbits lying apparently between 10.7 and 16.0 ml per kg, although one of 3 given the dosage of 7.1 ml per kg died, as did one of 3 given 4.7 ml per kilogram. The corresponding value for rats was greater than 24 ml per kg (Table 5).

Sample ML0-6405 induced no immediate signs of intoxication. Later, lethargy and seepage of oil about the anus were noted, the latter sign being more noteworthy in the case of rats. Occasionally, ataxia and dyspnea preceded a state of coma. In a few instances, rabbits exhibited convulsions following the administration of a single dose of the aviation gasoline (MLF-6400).

All rabbits and rats were subjected to post-mortem examination, and the viscera from representative animals were examined microscopically. The histologic alterations induced by lethal doses of formulations ML0-6405 and MLF-6400 consisted of diffuse degeneration of the livers, kidneys and brains, and edema and hyperemia of the lungs. The viscera of survivors were normal.

The Toxicity of the Materials When Maintained in Contact with the Skin of Rabbits for Twenty-four Hours.

The data relevant to samples A to J, when maintained in contact with the intact and abraded skin over the period of 24 hours, are given in Tables 6 and 7, respectively. The minimum lethal dosages which emerged from

the severe conditions of the procedure employed, have been listed in Table 8. The minimum lethal dose of sample A on the intact skin was greater than 0.39 and less than 0.60 ml per kg (Table 6), whereas, on the abraded skin it lay between 0.25 and 0.39 ml per kg (Table 7).

Samples F and H (commercial tricresyl phosphates) were about equally toxic when maintained in contact with the intact skin (Table 6), and both were somewhat less toxic when the skin was abraded. This apparent anomaly, encountered also in the experiments on samples C and J, but not in those on samples A, B, D, E and G, probably derives from the variability among animals, in which case it has resulted from the use of groups of but 3 rabbits. On the other hand, it might well be, as has been found to be the case from time to time, an expression of the inhibitory influence of injury upon percutaneous absorption. The data indicate that the intact skin is penetrated with ease. The other commercial samples (B, C, D, E and G) were slightly to moderately less toxic than samples F and H, samples C and G being lethal only when the dosage was 1.25 ml per kg, while sample B required 1.6 ml per kg, and samples D and E, 3.2 ml per kg to kill (Tables 6 and 8).

As when given by stomach tube, the xylenol phosphates were less toxic than the commercial tricresyl phosphates, when maintained undiluted on the intact skin of rabbits. Six milliliters of sample J per kg killed 1 of 3 rabbits, but the dosage of 9.4 ml of sample I per kg was tolerated by each of 3 rabbits (Tables 6 and 8).

None of the materials induced any change in the gross appearance of the skin although certain minor alterations (described below) were observed microscopically.

The application of a lethal dose of any one of samples A to J upon either the intact or abraded skin of rabbits, induced diffuse degenerative changes of the brain, liver, and kidneys, and hyperemia of the other viscera. Of the surviving animals, only those that had been subjected to contact with samples I or J exhibited degenerative changes in the liver and kidneys; the remaining viscera were unaltered. The skin of survivors as well as of those that died exhibited varying degrees of mild inflammation and in a few instances, focal acanthosis and slight hyperkeratosis, which disappeared 2 to 3 weeks after the exposure.

The pertinent data relating to contact of MLF-6400 and MLO-6405 with the skin are given in Table 9. Although the aviation gasoline (MLF-6400) was less toxic than formulation MLO-6405, when administered orally to rabbits (Table 5), the reverse was the case when the materials were maintained for 24 hours in contact with the skin. Three of 6 rabbits died following contact of their abraded skin with the dosage of 9.4 ml of MLF-6400 per kilogram. Corresponding contact of the abraded skin of 3 rabbits with the dosage of 6.0 ml per kg, and of 3 others with 3.2 ml per kg, induced no fatalities (Table 9). When MLF-6400 was maintained upon the intact skin of each of 3 rabbits in the dosage of 9.4 ml per kg, one of them died. Similar results were obtained when the dosage of 6.0 ml per kg was maintained in contact with the intact skin, but each of 3 survived the dosage of 3.2 per kilogram. Without exception, rabbits survived the dosage of 9.4 ml of MLO-6405 per kg, whether the skin was intact or abraded. These results indicate that gasoline promotes or fails to hinder the percutaneous absorption of tricresyl phosphate, to a considerably greater extent than does the mixture in MLO-6405, since the concentration of this compound in the latter (5 per cent) was far greater than it was in the samples of gasoline (5 ml per gallon). On the other hand, the extent of the damage to the skin induced by these materials, as well as the intrinsic absorbability and toxicity of the two vehicles, must be taken into account in the final result, and these experiments, as carried out, do not provide for such a comparison. Contact of the skin of the animals with MLF-6400 resulted in some erythema, and in fissuring and scaling of the skin, whereas contact with MLO-6405 occasionally gave rise only to dryness of the skin of an occasional animal. Microscopic examination of the viscera of the animals subjected to contact with formulation MLO-6405, on either intact or abraded skin, disclosed no alterations of the tissues. The skin exhibited slight inflammatory changes of low grade. Rabbits subjected to cutaneous contact with formulation MLF-6400 sustained diffuse degenerative changes in the brain, liver and kidneys. Sublethal amounts of the material, when applied upon the intact or abraded skin, induced slight degenerative changes in the liver. The areas of contact in the skin of rabbits that died were involved in acute inflammatory reaction. These changes in the skin of the survivors were less severe.

The Toxicity of the Materials When Applied Intermittently upon the Skin of Rabbits Over Prolonged Periods of Time.

Tables 10 and 16 present the data obtained when each of the 7 undiluted samples (samples B to H) of commercial tricresyl phosphate was kept for 2 hours upon the intact skin of separate groups of female rabbits on 5 days per week over a period of 10 weeks. Data obtained on controls, which were not subjected to contact with the materials under test, but were handled otherwise in the same manner as were test animals, are given in Table 18.

Each of 3 rabbits died, when subjected repeatedly, as indicated above, to contact with the unit dosage of 2.0 ml of tricresyl phosphate, sample B, as did also 4 rabbits so subjected to contact with 1.0 ml died (Table 10). The dosage of 0.50 ml was lethal to 1 of 3 rabbits, whereas 3 of 4 tolerated the dosage of 0.25 ml.

Corresponding dosages of 5.0 ml and 2.0 ml of tricresyl phosphate, sample C, were lethal to the two respective groups of 3 rabbits (Table 11) so tested. However, dosages of 1.0 or of 0.50 ml, similarly applied, resulted in the death of but 1 rabbit in each group of 3, while the dosage of 0.25 ml was tolerated by all of a group of 3 rabbits.

Each of 3 rabbits subjected to contact with the unit dosage of 2.0 ml of tricresyl phosphate, sample D, died, as did 3 others tested at the dosage level of 1.0 ml (Table 12), but only 1 of 3 and 1 of 4 died when the dosages were 0.50 and 0.25 ml, respectively. Similar results were obtained in the case of tricresyl phosphate, sample E (Table 13), and an additional group of 3 rabbits died when the level of dosage was 5.0 ml.

Rabbits in groups of 3 (Table 14) were subjected to contact with sample F at levels of dosage of 2.0, 1.0, 0.50 and 0.25 ml, respectively. Only 1 of 3 given the dosage of 0.50 survived.

Sample G killed all members of the test group at the dosage levels of 2.0, 1.0 or 0.50 ml, but that of 0.25 ml was tolerated by all of 3 rabbits (Table 15).

All of 17 rabbits died, when subjected to contact with sample H at levels of dosage ranging from 0.25 to 5.0 ml (Table 16).

The observed signs of intoxication in fatally poisoned animals related to the central nervous system and were characterized by inability to hold the head erect, ataxia and tremors. The skin of the animals was unaltered.

The average length of survival of the animals, expressed in terms of the numbers of unit doses of the respective materials applied, have been tabulated in Table 19 and plotted in Figure 8.

As indicated in the observations on the immediate toxicity of the materials, samples F and H were more toxic than the other grades of commercial tricresyl phosphate.

The lethal effects of multiple applications of samples B to H were associated with microscopic evidences of degenerative changes in the brain, liver and kidneys; the viscera of animals subjected to contact with sample C were also demonstrably hyperemic. Animals that survived the multiple applications of samples E to H had slight degenerative changes in their livers and kidneys, but the viscera from corresponding animals subjected to contact with samples B to D were unaltered. Samples B to H induced some degree of local inflammation, which in general was more severe in the skin of the animals that died than in that of survivors. Contact with lethal dosage levels of sample B and of samples D to H induced slight microscopic evidences of hyperkeratosis and acanthosis, and parakeratosis was seen in those subjected to contact with samples G and H. Some ulceration of the skin resulted from contact with samples E and F. Among the survivors subjected to contact with samples B to H slight inflammatory changes persisted, while samples G and H had induced some degree of residual keratosis of the skin. The skin and viscera of the control animal were unaltered.

When undiluted formulations MLF-6400 and MLO-6405 were applied upon the intact skin of rabbits and permitted to remain thereon for 2 hours on each of 50 days over a period of 10 weeks, the lesser of the unit dosages listed below was tolerated while the greater one resulted in some incidence of mortality (Table 17).

MLF-6400	1.0 - 2.0 ml (0.34 - 0.93 ml/kg)
MLO-6405	1.0 - 2.0 ml (0.36 - 0.87 ml/kg)

The average number of periods of contact to which the animals were subjected before they died or were killed are given in Table 19. The animals that died as the result of contact with the gasoline (MLF-6400) at the relatively high level of dosage lived longer, i.e., were subject to a larger total volume of the sample, than did those subjected to contact with formulation MLO-6405. Formulation MLO-6405 was tolerated to the extent comparable to that of some of the commercial grades of tricresyl phosphate (Table 19).

The multiple applications of small volumes of MLF-6400 and MLO-6405 induced dryness and scaliness of the skin, while larger volumes induced erythema, petechial hemorrhages and fissuring. Contact with sample MLO-6405 over a single period of 24 hours, as indicated previously, altered the skin but little, whereas multiple applications of this material induced lesions which may have accelerated percutaneous absorption. These lesions, sustained by animals subjected to relatively high levels of dosage, consisted of superficial fissures and a scattering of petechial hemorrhages. Some dryness and scaliness of the skin, were associated with the lowest level of dosage. This factor of cutaneous injury may account for the difference in the outcome of the experiments, in the one of which MLO-6405 and MLF-6400 were applied in one dose without causing injury, and in the others, just described, were applied repeatedly, with resultant injury. The relative toxicity of the two materials appears to have been reversed, the former being the more toxic under the conditions of repetitive applications. Despite the difference in the composition of these two samples, however, their effects, in terms of the pathological changes induced in the tissues of the animals, were the same. The rabbits that died had sustained degenerative changes in the brain, heart, liver, and kidneys, and those that survived had comparable, residual, degenerative lesions in the liver and kidneys. Since these changes were of a non-specific type, they do not identify the compound or compounds absorbed in either instance.

The Toxicity of the Vapor of Tricresyl Phosphate.

A group of animals, consisting of a cat, 2 guinea pigs, 5 mice, 2 rabbits and 3 rats, survived when subjected to the inhalation of air laden with 61.5 micrograms of tricresyl phosphate per liter, for 7 hours on each of 8 days over the period of 10 days (Table 20). The changes in the weights of the animals were small and transitory, and no

signs of intoxication were observed. The viscera of the rats and mice were normal, while the lesions observed in those of the rabbits and guinea pigs were due to infectious processes unrelated to the experiment.

The Toxicity of Products Obtained Through Contact of Tricresyl Phosphate with Inconel at Either 1250° or 1050°F.

Data relative to the duration of the exposure, and the fate, of animals that were subjected to the inhalation of the products provided by the contact of tricresyl phosphate with Inconel at 1250° or 1050°F. are given in Table 21. The data concerning mortality among all of the groups of animals employed in these experiments have been summarized in Table 22.

Careful examination of the data demonstrates certain general facts. It is apparent that, in terms of over-all mortality, guinea pigs and mice were more susceptible than were rats or rabbits, to the materials in the atmosphere. The fog formed at the lower temperature appeared to be slightly more toxic than that generated at the higher one (Table 22), but regardless of the factor of temperature, all animals survived when subjected to the fog for 20 minutes. In general, as the length of exposure increasecd, the incidence of mortality became greater.

In order to ascertain the effect of different rates of delivery of the liquid into the Inconel tube, while the rate of flow of air through the tube was kept constant (20 liters per minute), tricresyl phosphate was delivered at two different rates (Table 21). The mortality associated with delivery at the lower rate was relatively less in some instances.

Although in these experiments the fogs filled the chamber to such an extent that it was impossible clearly to observe the animals throughout the period of exposure, a few signs of intoxication could be observed. The fogs were immediately irritating to the mucous membranes of the animals, as evidenced by their closure of the eyes, twitching of the nose, crying, coughing and sneezing. Labored respiration and irritation of the superficial tissues of the eyes appeared later. Dyspnea appeared after an hour. Whenever cats were exposed, excessive salivation was evident, and frequently long strands of thick mucous drooled from the mouth. Black soot covered the hair of animals exposed to fogs generated at the higher temperature (1250°F.).

Animals that died during or shortly after a period of exposure to the fog of tricresyl phosphate had sustained extensive pulmonary congestion and, in occasional animals, foci of pulmonary hemorrhage. Many also had foci of black pigment, that appeared to be carbon, distributed throughout the lung tissues. Microscopically, the lesions consisted of marked hyperemia of the lung, common to all exposed animals examined, and intra-alveolar hemorrhage and edema in the lungs of many of those. The carbon pigment was distributed chiefly within the bronchi and, in some instances, it completely occluded their lumens. In a few of the guinea pigs and rabbits that died during the period of repetitious exposure, there was, in addition to the more common signs of chemical irritation, acute purulent bronchitis, apparently a reaction to bronchial irritation. Fatally poisoned animals exhibited parenchymatous degeneration of the liver, kidneys and brain, with regularity, in addition to the pulmonary lesions. The viscera of animals that died 7 to 10 days after brief periods of exposure were the sites of pulmonary lesions similar to those described above, except that there was more phagocytosis of carbon particles. In animals that survived and were killed after intervals varying from 1 week to 2 months, there were no significant lesions in viscera other than the lungs. The lungs of a cat, which had been subjected to 2 periods of exposure to the fog of tricresyl phosphate and its decomposition products, were the site of interstitial pneumonitis and profuse intraluminal bronchial deposits of black granular material. A guinea pig, killed 22 days after 2 periods of such exposure, was found to have numerous histiocytes loaded with carbon pigment in the lungs, as well as a severe interstitial pneumonitis. Other species of animals had similar lesions of interstitial pneumonitis. However, animals that had been subjected to the shortest periods of exposure, and animals that had survived and were killed 28 days or longer after a period of exposure, exhibited normal viscera.

Although only a part of the materials metered into the Inconel tube could be accounted for in the air in the chamber, some tentative statements concerning the composition of the air may be made. Although large amounts of soot were formed, no attempts were made to measure the amounts of free carbon or carbon dioxide. These and perhaps other unidentified materials were present in greater quantities when the decomposition was effected at the higher temperature.

More of the undecomposed material and more free cresols were present in the chamber when the temperature of the Inconel tube was relatively low. The values reported (Table 23) for tricresyl phosphate, as determined by hydrolysis and colorimetric determination of the resultant cresols, may be somewhat low, since results several fold higher could be obtained by measuring at 264 mμ the ultraviolet absorption of alcoholic solutions of spot samples collected in an evacuated balloon. Determinations by this means may, however, have given high results, since filtration failed to remove a slight smoky turbidity.

DISCUSSION

Smith, Elvove and Frazier found rabbits susceptible to the oral administration of one dose of triorthocresyl phosphate in alcohol; 1 of 4, 1 of 4, 3 of 5, and 5 of 5 rabbits died following the administration of 50, 75, 100, and 150 mg per kg, respectively. Gross and Gross gave triorthocresyl phosphate mixed with peanut oil and meal, by mouth, to rabbits; a group of 8, each given 100 mg per kg died, whereas 1 of 5 died when the dosage was 50 mg per kilogram. Smith, Engle and Stohlman found that rats tolerated large doses of triorthocresyl phosphate.

A limited series of observations made by Scientific Associates of St. Louis, for Monsanto Chemical Company (letter of December 4, 1953, by F.M. Younger), indicate that male rabbits tolerate a large dosage of undiluted commercial tricresyl phosphate. Eight male rabbits were given, respectively, 25.0, 20.0, 15.0, 15.0, 10.0, 7.5, 5.0 and 2.0 ml of tricresyl phosphate per kilogram. Six of the 8 survived, whereas the rabbit given the dosage of 25.0 ml per kg, and 1 of the 2 given 15.0 ml per kg, died after having developed severe diarrhea. Nelson gave one oral dose of one or the other of two commercial preparations of tricresyl phosphate to each of a series of rabbits and rats (sex not mentioned); the lethal dosages (LD_{50}), of these preparations were 3.0 and 0.6 g per kg, respectively, for rabbits, and 15.0 and 3.2 g per kg, respectively, for rats. Other unpublished data relevant to the oral administration of commercial tricresyl phosphate to rabbits have shown that the results, in terms of mortality, are variable, but in no other instance have these animals been found to be as resistant to fatal poisoning as were the male rabbits employed in the experiments of Scientific Associates, as referred to above.

Although the toxicity of none of the six isomers of xylenol phosphate (1 hydroxy 2,3-dimethyl benzene, 2,4- 2,5- 2,6-, 3,4- and 3,5-dimethyl) has been reported in toxicological literature, it appears that mixed xylenol phosphates, such as those involved in these experiments, would not, by their presence therein, enhance the toxicity of commercial tricresyl phosphates.

Formulation ML0-6405, which differed from PRL-3161 only in containing triorthocresyl phosphate instead of the mixed tricresyl phosphates, was apparently more toxic for rabbits than either PRL-3161 or its principal ingredient, di-2-ethylhexyl sebacate. Rats, which are more resistant than rabbits to the toxic effects of triorthocresyl phosphate, manifested no apparent difference in susceptibility to these two formulations when each was administered in one oral dose.

The presence of triorthocresyl phosphate enhanced the cumulative toxicity of ML0-6405, over that of the preparation containing the mixed isomers of tricresyl phosphate, under the conditions associated with multiple applications of the materials upon the skin of rabbits. It was shown in the report of April 29, 1954, on "The Immediate and Cumulative Toxicity of Synthetic Lubricants MRD-52-8 and MRD-52-9" (unused and used WS-2211), prepared for The Standard Oil Development Company by The Kettering Laboratory and made availabe to WADC, that rabbits survived multiple cutaneous applications of 2.0 ml of either MRD-52-8 or MRD-52-9, which consisted essentially of di-2-ethylhexyl sebacate, small amounts of a complex ester (ED35) and mixed tricresyl phosphates, and a small amount of phenothiazine. However, the corresponding dosage of ML0-6405, which consisted principally of di-2-ethylhexyl sebacate and triorthocresyl phosphate, was always lethal.

Since the commercial grades of tricresyl phosphate possess a cumulative action, due precaution should be taken to prevent their ingestion or contact with the skin. In case of contact with the skin they should be removed rapidly by generous flushing with water.

Scientific Associates reported to Monsanto Chemical Company that 10 rats survived exposure for 10 hours to the fumes (in the concentration of 2.8 mg of tricresyl phosphate per liter of air), which resulted from heating tricresyl phosphate to temperatures ranging from 250°C to 275°C (482°F. to 527°F.). They also reported that 3 male rabbits survived when exposed

for 7 hours on 3 consecutive days to the fumes formed from tricresyl phosphate (1.91 mg of TCP per liter) heated to these temperatures. These fumes induced some irritation of the membranes of the nose and eyes of rats and rabbits together with evidences of labored respiration on the part of the rabbits. However, Nelson reported a high incidence of mortality among rabbits (sex not mentioned) when the head only was exposed to a mist (particles less than 5 microns in diameter), generated by atomizing tricresyl phosphate heated to 200°F. The concentrations of tricresyl phosphate in the mist ranged from 1.3 to 2.2 mg per liter, and the duration of exposure ranged from 3 hours on 1 day to 27 hours over the period of 18 days.

Although the products resulting from the decomposition of commercial tricresyl phosphate at 1050° or 1250°F. are toxic when inhaled, their toxicity seems to be no greater than that of the decomposition products of a typical paraffinic lubricating oil at 800°F., on which observations were made for Monsanto Chemical Company. A group of animals consisting of 5 mice, 2 rats, 2 guinea pigs and 3 rabbits (experiment 7) survived exposure for 1 hour to the fog formed by dropping 0.072 ml of tricresyl phosphate per minute into an Inconel tube (at 1250°F.) through which air was flowing at the rate of 20 liters per minute (Tables 21 and 23). In terms of the tricresyl phosphate delivered, this concentration was equivalent to 4.25 mg per liter $\left(\frac{0.072 \text{ ml}}{\text{min}} \times \frac{1.18 \text{ g}}{\text{ml}} \times \frac{1000 \text{ mg}}{\text{g}}\right) \Big/ 20 \frac{\text{l.}}{\text{min}}$. In comparison, only 1 rabbit from a group consisting of 5 mice, 4 rats, 2 guinea pigs and 4 rabbits died when exposed for 1 hour to the fog formed by dropping the paraffinic hydrocarbon fluid at the rate of 114 mg per minute into an Inconel tube maintained at 800°F., through which air was flowing at the rate of 30 liters per minute. This was equivalent to 3.8 mg of the fluid per liter of air $\left(\frac{114 \text{ mg/min}}{30 \text{ l./min}}\right)$. In another 1-hour experiment (No. 4), in which tricresyl phosphate was decomposed at 1250°F., 2 guinea pigs and a rabbit died, but 5 mice, 2 rats and 2 rabbits survived. In this experiment the fog was formed by delivering the phosphate at the rate of 0.233 ml per minute, which was equivalent to 13.75 mg per liter $\left(\frac{0.233 \text{ ml}}{\text{min}} \times \frac{1.18 \text{ g}}{\text{ml}} \times \frac{1000 \text{ mg}}{\text{g}}\right) \Big/ 20 \frac{\text{l.}}{\text{min}}$. In another 1-hour experiment,

in which the paraffinic fluid was decomposed at 800°F., 2 of 4 rats and 2 of 4 rabbits died, but 2 guinea pigs and 5 mice survived. In this instance the concentration was equivalent to 7.1 mg per liter ($\frac{214 \text{ mg/min}}{30 \text{ l./min}}$).

In the concentration of 1.3 to 2.0 mg per liter (1174 to 1805 ppm), carbon monoxide caused no significant signs of intoxication in mice exposed for 6 hours by Chernov and Liberman, although 20 to 40 per cent of a group of mice died as a result of exposure for a like period to the concentration of 6 mg of carbon monoxide per liter (5416 ppm). Since the mice in our experiments died under conditions which were much less severe with respect to carbon monoxide, it is evident that this compound by itself was not responsible for their deaths. Comparable information with respect to other species of animals is not readily available, although Moss, Jackson and Seiberlich have reported that carbon monoxide in the concentration of 5,000 ppm was lethal for a rat in 30 minutes.

Data pertaining to the toxicity of cresols in air are not available; however, Deichmann, Kitzmiller and Witherup found that 20 periods of exposure, each of 7 hours, to the vapor of phenol in the concentration of 0.1 to 0.2 mg per liter in air, were required to kill some of the guinea pigs subjected to such exposure. Rabbits and rats survived after much longer periods of exposure. It does not appear that the deaths reported herein are directly attributable to the vapor of cresol.

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APPENDIX

The methods used and the results obtained on the materials described in this report were given in greater detail in the following interim reports:

March 11, 1953 - The Toxicity of the Fog Formed by Dropping Tricresyl Phosphate upon the Surface of a Heated Inconel. (Prepared for the Monsanto Chemical Company by The Kettering Laboratory.)

May 11, 1953 - The Immediate Toxicity of Certain Aliphatic Esters.

May 3, 1954 - The Cumulative Toxicity of MLF-6400 and MLO-6405 When Maintained in Contact with the Skin of Rabbits.

Table 1

Fate of Rabbits Given One Oral Dose of
Undiluted Tricresyl or Xylenol Phosphate

Dose ml/kg	Number Died/Number Given Dose				
	Sample A	Sample B	Sample C	Sample D	Sample E
16.0	-	-			
10.7	-	-			
7.1	-	-			
4.7	-	-		1/1	
3.2	-	2/2	3/3	1/1	2/3
2.1	-	3/3	1/3	3/3	2/3
1.4	-	1/3	1/3	3/3	1/3
0.94	-	1/3	1/3	1/3	0/3
0.62	-	0/3	1/3	1/3	1/3
0.42	-	0/3	0/3	0/3	0/3
0.28	3/3	-			
0.18	3/3	-			
0.12	2/3	-			

Table 1 (Continued)

Fate of Rabbits Given One Oral Dose of Undiluted Tricresyl or Xylenol Phosphate

Number Died/Number Given Dose				
Sample F	Sample G	Sample H	Sample I	Sample J
			1/3	1/4
			1/3	0/4
			1/3	0/4
			1/3	1/4
1/1	1/1		2/3	0/4
1/1	3/3	1/1	0/3	0/4
2/2	2/3	3/3	0/3	
3/3	3/3	3/3	0/1	
3/3	1/3	3/3	0/1	
3/3	0/3	3/3	0/1	
3/3	0/3	1/3	0/1	
2/3		0/3	0/1	
0/3		0/3		

Table 2

Fate of Rabbits Given One Oral Dose of a Solution of Tricresyl or Xylenol Phosphate

(Given as a 5 per cent V/V solution in peanut oil unless otherwise stated)

Dosage ml/kg	Number that Died/Number Given the Dosage	
	Sample A	Sample C
3.2	-	-
2.1	-	-
1.4	1/1	-
0.94	1/1	1/3
0.62	1/1	1/3
0.42	1/1	1/3
0.28	2/2	0/3
0.18	2/2	0/1
0.12	2/3	0/1
0.080	1/3	0/1
0.055	0/3	0/1
0.037	0/3	-

Table 2 (Continued)

Fate of Rabbits Given One Oral Dose of a Solution of Tricresyl or Xylenol Phosphate

(Given as a 5 per cent V/V solution in peanut oil unless otherwise stated)

Number that Died/Number Given the Dosage		
Sample D	Sample I	Sample J
-		1/3 (1)
2/3		1/3 (1)
1/3	1/3	0/3 (1)
1/3	1/3	0/2 (1)
1/3	1/3	0/1
0/1	2/3	0/1
0/1	0/3	-
0/1	0/3	-
0/1	0/1	-
0/1	0/1	-
0/1	0/1	-
-	-	-

(1) Given as a 10 per cent V/V solution in peanut oil.

Table 3

Fate of Rats Given One Oral Dose of
Undiluted Tricresyl or Xylenol Phosphate

Dose ml/kg	Number that Died/Number Given the Dosage				
	Sample A	Sample B	Sample C	Sample D	Sample E
16.0	1/4	0/4	0/4	0/4	0/4
10.7	1/4	0/4	1/4	1/4	0/4
7.1	1/4	0/4	0/4	0/4	0/4
4.7	1/4	0/4	0/1	0/1	
3.2	0/4	0/4	0/1	0/1	
2.1	0/2	0/4	0/1	0/1	
1.4	0/3	1/4			
0.94	0/2	0/4			
0.62					
0.42					

Table 3 (Continued)

Fate of Rats Given One Oral Dose of Undiluted Tricresyl or Xylenol Phosphate

Number that Died/Number Given the Dosage				
Sample F	Sample G	Sample H	Sample I	Sample J
1/4	0/4	2/4	1/4	0/4
0/4	0/4	1/4	0/4	0/4
0/4	0/4	0/4	0/4	0/4
				-
				-
			0/2	0/2

Table 4

Summarized Data on the Toxicity of Various Samples of Tricresyl and Xylenol Phosphate When Given as One Oral Dose to Rabbits and Rats

Sample	Range of Minimum Lethal Dose; ml/kg		
	Given Undiluted		Given as a Solution in Peanut Oil
	Rabbits	Rats	Rabbits
J	10.7 -16.0 (1)	>16.0	1.4 -2.1
I	2.1 - 3.2	10.7-16.0	0.28-0.42
B	0.62- 0.94	>16.0	-
C	0.42- 0.62	7.1-10.7 (2)	0.28-0.42
D	0.42- 0.62	7.1-10.7 (2)	0.42-0.62
G	0.42- 0.62	>16.0	-
E	0.42- 0.62	>16.0	-
H	0.18- 0.28	7.1-10.7	-
F	0.12- 0.18	10.7-16.0	-
A	<0.12	3.2- 4.7	0.055-0.080

(1) One of 4 rats, each given the dosage of 4.7 ml/kg died.

(2) One of 4 rats, each given the dosage of 10.7 ml/kg, died, but 4 rats, each given the dosage of 16.0 ml/kg, survived.

Table 5

Fate of Rabbits and Rats Given One Oral Dose of Either Formulation MLO-6405 or MLF-6400

Dose ml/kg	Number of Animals that Died/ Number given the Material			
	MLO-6405		MLF-6400	
	Rabbits	Rats	Rabbits	Rats
24.0	-	-	2/3	0/4
16.0	-	4/4	2/3	0/4
10.7	-	1/4	0/3	0/4
7.1	1/1	0/4	1/3	0/4
4.7	1/1	0/4	1/3	0/1
3.2	3/3	-	0/3	-
2.1	3/3	-	-	-
1.4	0/3	-	-	-
0.94	0/3	-	-	-

Table 6

The Immediate Toxicity of Various Samples of Tricresyl or Xylenol Phosphate When Maintained for Twenty-four Hours in Contact with the Intact Skin of Rabbits by the Sleeve Method of Draize, Woodard and Calvery

(applied undiluted)

Dose ml/kg	Number that Died/Number Given the Material				
	Sample A	Sample B	Sample C	Sample D	Sample E
9.4	-	2/3	1/3	4/6	-
6.0	-	2/3	3/3	-	2/2
3.2	3/3	1/3	0/6	1/3	2/3
1.6	1/3	1/3	2/6	0/3	0/3
1.25	1/3	0/5	3/6	-	-
0.60	1/3	0/3	0/6	-	-
0.39	0/3	-	-	-	-

Table 6 (Continued)

The Immedicate Toxicity of Various Samples of Tricresyl or Xylenol Phosphate When Maintained for Twenty-four Hours in Contact with the Intact Skin of Rabbits by the Sleeve Method of Draize, Woodard and Calvery

(applied undiluted)

Number that Died/Number Given the Material				
Sample F	Sample G	Sample H	Sample I	Sample J
-	-		0/3	2/3
-	-		-	1/3
3/3	1/3	2/3	-	0/3
3/3	1/3	2/3	-	-
2/3	2/3	0/3	-	-
1/3	0/3	2/3	-	-
0/3	-	o/3	-	-

Table 7

The Immediate Toxicity of Various Samples of Tricresyl or Xylenol Phosphate When Maintained for Twenty-four Hours in Contact with the Abraded Skin of Rabbits by the Sleeve Method of Draize, Woodard and Calvery

(applied undiluted)

Dose ml/kg	Number that Died/Number Given the Material				
	Sample A	Sample B	Sample C	Sample D	Sample E
9.4	-	-	1/3	3/3	-
6.0	-	1/3	1/3	-	2/2
3.2	3/3	1/3	1/3	1/3	1/3
1.6	3/3	1/3	0/3	1/3	1/3
1.25	3/3	1/3	0/3	0/3	0/3
0.60	1/3	0/3	-	-	-
0.39	1/3	-	-	-	-
0.25	0/3	-	-	-	-

Table 7 (Continued)

The Immediate Toxicity of Various Samples of Tricresyl or Xylenol Phosphate When Maintained for Twenty-four Hours in Contact with the Abraded Skin of Rabbits by the Sleeve Method of Draize, Woodard and Calvery

(applied undiluted)

Number that Died/Number Given the Material				
Sample F	Sample G	Sample H	Sample I	Sample J
-	-	-	1/3	3/3
-	-	-	1/3	0/3
3/3	3/3	3/3	0/3	0/3
2/3	1/3	2/3	-	-
2/3	1/3	2/3	-	-
0/3	1/3	0/3	-	-
-	0/3	-	-	-
-	-	-	-	-

Table 8

Summarized Data on the Immediate Toxicity of Various Samples of Tricresyl and Xylenol Phosphates When Maintained in Contact with the Intact or Abraded Skin of Rabbits by the Method of Draize, Woodard and Calvery

(applied undiluted)

Sample	Range of Minimum Lethal Dose; ml/kg	
	Intact Skin	Abraded Skin
I	>9.4	3.2 -6.0
J	3.2 -6.0	6.0 -9.4
D	1.6 -3.2	1.25-1.6
E	1.6 -3.2	1.25-1.6
B	1.25-1.6	0.6 -1.25
C	0.60-1.25	1.6 -3.2
G	0.60-1.25	0.39-0.60
F	0.39-0.60	0.60-1.25
H	0.39-0.60	0.60-1.25
A	0.39-0.60	0.25-0.36

Table 9

The Immediate Toxicity of Tricresyl Phosphate in Gasoline (MLF-6400) Or in a Formulation of Di-2-Ethylhexyl Sebacate (MLO-6405) When Maintained for Twenty-four Hours in Contact with the Skin of Rabbits by the Sleeve Method of Draize, Woodard and Calvery

(applied undiluted)

Material	Condition of Skin	Number of Rabbits that Died/ Number Given the Material		
		9.4 ml/kg	6.0 ml/kg	3.2 ml/kg
MLO-6405	Abraded	0/3	-	-
	Intact	0/3	-	-
MLF-6400	Abraded	3/6	0/3	0/3
	Intact	1/3	1/3	0/3

Table 10

The Fate of Rabbits When Tricresyl Phosphate (Sample B) Was Applied Repetitively Upon Their Skin

Daily Dosage of Tricresyl Phosphate			Number of Doses per Animal	
	ml/kg			
ml	Mean (1)	Range	Mean	Range
2.0	0.83 (D)	0.72 - 0.98	22.3	15 - 31
1.0	0.41 (D)	0.31 - 0.62	35.8	9 - 50
0.50	0.20 (D)	0.19 - 0.21	14.0	14
	0.19 (L)	0.18 - 0.20	50.0	50
0.25	0.13 (D)	0.11 - 0.17	19.0	19
	0.09 (L)	0.07 - 0.11	50.0	50

(1) The mean daily dosage is the mean of the means for each animal.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

The Fate of Rabbits When Tricresyl Phosphate (Sample B) Was Applied Repetitively Upon Their Skin

Average Initial Weight kg	Weight Change During Period of Exposure (2), Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.482	-30.0	-28.1 to -31.7	3/3
2.493	-23.9	-15.0 to -39.3	4/4
2.515	-22.7	-22.7	1/3
2.682	- 2.5	- 9.0 to + 4.1	
2.350	-40.2	-40.2	1/4
2.165	+46.9	+40.0 to +51.5	

(1) The mean daily dosage is the mean of the means for each animal.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.

L = Based upon rabbits that survived.

Table 11

The Fate of Rabbits When Tricresyl Phosphate (Sample C) Was Applied Repetitively Upon Their Skin

Daily Dosage of Tricresyl Phosphate			Number of Doses per Animal	
	ml/kg			
ml	Mean (1)	Range	Mean	Range
5.0	2.0 (D)	1.82 - 2.20	4.3	3 - 5
2.0	0.91 (D)	0.73 - 1.32	17.0	12 - 24
1.0	0.38 (D)	0.35 - 0.47	42.0	42
	0.40 (L)	0.32 - 0.47	50	50
0.50	0.20 (D)	0.17 - 0.25	35.0	35
	0.19 (L)	0.16 - 0.24	50.0	50
0.25	0.09 (L)	0.07 - 0.13	50.0	50

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 11 (Continued)

The Fate of Rabbits When Tricresyl Phosphate (Sample C) Was Applied Repetitively Upon Their Skin

Average Initial Weight kg	Weight Change During Period of Exposure (2), Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.671	-20.9	-17.1 to -25.2	3/3
2.525	-32.0	-25.3 to -39.7	3/3
2.582	-17.5	-17.5	1/3
2.239	+25.4	+ 7.4 to +43.3	
2.027	- 7.5	- 7.5	1/3
2.227	+44.0	+31.5 to +52.4	
2.518	+27.1	+16.6 to +34.5	0/3

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to t he last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 12

The Fate of Rabbits When Tricresyl Phosphate (Sample D) Was Applied Repetitively Upon Their Skin

Daily Dosage of Tricresyl Phosphate			Number of Doses per Animal	
	ml/kg			
ml	Mean (1)	Range	Mean	Range
2.0	0.97 (D)	0.73 - 1.25	4.7	4 - 6
1.0	0.44 (D)	0.32 - 0.55	16.0	3 - 26
0.50	0.22 (D)	0.20 - 0.24	39.0	39
	0.20 (L)	0.17 - 0.27	50.0	50
0.25	0.13 (D)	0.11 - 0.16	13	13
	0.10 (L)	0.07 - 0.11	50.0	50

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 12 (Continued)

The Fate of Rabbits When Tricresyl Phosphate (Sample D) Was Applied Repetitively Upon Their Skin

Average Initial Weight kg	Weight Change During Period of Exposure (2), Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.326	-25.3	-15.4 to -35.4	3/3
2.289	-14.7	- 1.7 to -27.7	3/3
2.250	-28.2	-28.2	1/3
2.022	+44.8	+35.3 to +54.2	
2.100	-35.8	-35.8	1/4
2.207	+53.6	+50.1 to +59.7	

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 13

The Fate of Rabbits When Tricresyl Phosphate (Sample E) Was Applied Repetitively Upon Their Skin

Daily Dosage of Tricresyl Phosphate			Number of Doses per Animal	
	ml/kg			
ml	Mean (1)	Range	Mean	Range
5.0	2.43 (D)	2.09 - 3.93	5.0	4 - 6
2.0	0.80 (D)	0.65 - 1.00	19.3	12 - 27
1.0	0.43 (D)	0.31 - 0.79	27.3	16 - 50
0.50	0.21 (D)	0.20 - 0.22	24.0	24
	0.21 (L)	0.16 - 0.26	50.0	50
0.25	0.12 (D)	0.11 - 0.14	9.0	9
	0.10 (L)	0.07 - 0.14	50.0	50

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 13 (Continued)

The Fate of Rabbits When Tricresyl Phosphate (Sample E) Was Applied Repetitively Upon Their Skin

Average Initial Weight kg	Weight Change During Period of Exposure (2), Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.344	-29.0	-21.7 to -43.3	3/3
2.626	-19.9	- 9.1 to -38.9	3/3
2.452	-13.2	-43.5 to +15.0	3/3
2.401	- 6.7	- 6.7	1/3
2.169	+37.4	+35.8 to +38.9	1/4
2.330	-33.0	-33.0	
2.578	+35.3	+23.7 to +49.3	

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 14

The Fate of Rabbits When Tricresyl Phosphate (Sample F) Was Applied Repetitively Upon Their Skin

Daily Dosage of Tricresyl Phosphate			Number of Doses per Animal	
	ml/kg			
ml	Mean (1)	Range	Mean	Range
2.0	0.83 (D)	0.63 - 1.12	2.7	2 - 3
1.0	0.46 (D)	0.33 - 0.50	6.0	3 - 12
0.50	0.22 (D)	0.18 - 0.28	22.0	6 - 38
	0.20 (L)	0.18 - 0.23	50.0	50
0.25	0.13 (D)	0.11 - 0.17	16.7	4 - 23

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 14 (Continued)

The Fate of Rabbits When Tricresyl Phosphate (Sample F) Was Applied Repetitively Upon Their Skin

Average Initial Weight kg	Weight Change During Period of Exposure (2), Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.656	-15.8	-13.7 to -17.5	3/3
2.454	-20.1	-10.7 to -24.8	3/3
2.546	-31.4	-27.5 to -35.2	2/3
2.434	+ 5.3	+ 5.3	
2.027	-26.2	-22.0 to -30.5	3/3

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 15

The Fate of Rabbits When Tricresyl Phosphate (Sample G) Was Applied Repetitively Upon Their Skin

Daily Dosage of Tricresyl Phosphate			Number of Doses per Animal	
	ml/kg			
ml	Mean (1)	Range	Mean	Range
2.0	1.00 (D)	0.92 - 1.06	4.3	4 - 5
1.0	0.57 (D)	0.43 - 0.86	13.0	9 - 21
0.50	0.25 (D)	0.20 - 0.35	15.3	12 - 19
0.25	0.10 (L)	0.08 - 0.13	50	50

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 15 (Continued)

The Fate of Rabbits When Tricresyl Phosphate (Sample G) Was Applied Repetitively Upon Their Skin

Average Initial Weight kg	Weight Change During Period of Exposure (2), Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.194	-29.6	-25.5 to -33.5	3/3
2.114	-39.2	-20.2 to -47.9	3/3
2.191	-31.9	-18.4 to -43.4	3/3
2.183	+41.4	+22.6 to +53.7	0/3

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.
L = Based upon rabbits that survived.

Table 16

The Fate of Rabbits When Tricresyl Phosphate (Sample H) Was Applied Repetitively Upon Their Skin

Daily Dosage of Tricresyl Phosphate			Number of Doses per Animal	
	ml/kg			
ml	Mean (1)	Range	Mean	Range
5.0	1.97 (D)	1.78 - 2.19	1.7	1 - 2
2.0	0.85 (D)	0.78 - 0.92	2.0	2
1.0	0.49 (D)	0.38 - 0.72	4.6	3 - 7
0.50	0.23 (D)	0.18 - 0.28	4.7	4 - 6
0.25	0.11 (D)	0.08 - 0.16	34.3	25 - 45

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.

Table 16 (Continued)

The Fate of Rabbits When Tricresyl Phosphate (Sample G) Was Applied Repetitively Upon Their Skin

Average Initial Weight Kg	Weight Change During Period of Exposure (2), Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.614	- 8.1	- 4.5 to -11.2	3/3
2.445	-10.9	- 4.7 to -16.5	3/3
2.287	-23.0	-12.4 to -34.9	5/5
2.439	-20.8	-15.5 to -30.3	3/3
2.534	-23.8	-13.8 to -36.7	3/3

(1) The mean daily dosage is the mean of the means for the individual animals.

(2) In the case of animals that died subsequent to the last period of contact, the loss during the few days after the final application has been included.

D = Based upon rabbits that died.

Table 17

The Fate of Rabbits When Undiluted MLF-6400 or MLO-6405 Was Applied Repetitively Upon Their Intact Skin

Material Applied	Daily Dosage of Material			Number of Doses per Animal	
		ml/kg			
	ml	Mean (1)	Range	Mean	Range
MLF-6400	5.0	1.97 (D)	1.46-2.44	20	1 - 41
		1.73 (L)	1.29-2.31	50	50
	2.0	0.93 (D)	0.87-0.97	42	42
		0.79 (L)	0.58-1.31	50	50
	1.0	0.34 (L)	0.28-0.44	50	50
MLO-6405	5.0	1.81 (D)	0.96-2.50	4.7	4 - 5
	2.0	0.87 (D)	0.64-1.27	12.3	5 - 20
	1.0	0.36 (L)	0.28-0.42	50	50

(1) The mean daily dosage is the mean of the means for the individual animals.

D = Based upon rabbits that died
L = Based upon rabbits that lived.

Table 17 (Continued)

The Fate of Rabbits When Undiluted MLF-6400 or MLO-6405 Was Applied Repetitively Upon Their Intact Skin

Average Initial Weight kg	Weight Change Percentage of Initial Weight		Incidence of Mortality
	Mean	Range	
2.662	+ 3.5	- 5.3 to +12.0	3/7
2.891	+14.1	- 8.6 to +30.2	
2.236	-10.6	-10.6	1/4
2.158	+53.4	+30.7 to +71.9	
2.456	+34.2	+26.2 to +43.9	0/3
2.692	-18.0	-11.5 to -22.2	3/3
2.868	-30.2	- 7.2 to -43.6	3/3
2.717	+20.5	+14.3 to +30.1	0/3

(1) The mean daily dosage is the mean of the means for the individual animals.

D = Based upon rabbits that died.
L = Based upon rabbits that lived.

Table 18

The Fate of Control Rabbits When Immobilized in Stocks, for Two Hours on Each of Five Days Per Week Over a Period of Ten Weeks

Number of Immobilizations per Animal	Average Initial Weight kg	Weight Change Percentage of Initial Weight		Incidence of Mortality
		Mean	Range	
50	2.494	+26.5	+19.2 to +43.9	0/8

Table 19

The Average Number of Periods of Contact Survived by Rabbits When Commercial Grades of Tricresyl Phosphate or Formulations MLF-6400 and MLO-6405 Were Maintained Upon Their Skin for Two Hours per Day on Five Days per Week

	Average Number of Doses Survived				
	Daily Dosage; ml				
Sample	5.0	2.0	1.0	0.50	0.25
C	4.3	17.0	47.3	45.0	50.0
B	-	22.3	35.8	38.0	42.3
E	5.0	19.3	27.3	41.3	39.8
D	-	4.7	16.0	46.3	40.8
G	-	4.3	13.0	15.3	50.0
F	-	2.7	6.0	31.3	16.7
H	1.7	2.0	4.6	4.7	34.3
MLF-6400	37.1	48.0	50.0	-	-
MLO-6405	4.7	12.3	50.0	-	-

Table 20

The Fate of Animals Exposed to the Vapor of a Commercial Grade (Monsanto) of Tricresyl Phosphate in Air

Concentration		Duration of Exposure Hours	Number of Animals that Died/ Number of Animals Exposed				
mg/l	ppm		Cat	Guinea Pigs	Mice	Rabbits	Rats
0.062	4.2	8 x 7.0	0/1	0/2	0/5	0/2	0/3

Table 21

Mortality Among Groups of Animals Subjected to the Fogs Formed by Dropping Tricresyl Phosphate into a Heated Inconel Tube

Temperature of Metal	Duration Hours	Mice	Rats	Guinea Pigs	Rabbits	Cats	Experiment No.
1250°F	2 x 7	5/5	2/2	1/2	1/3	0/1	15
	7.0	5/5	2/2	2/2	0/3	-	2
	3.5	3/5	0/2	2/2	0/3	-	1
	1.0	0/5	0/2	2/2	1/3	-	4
	0.33	0/5	0/2	0/2	0/3	-	6
	3.5 *	4/5	1/2	0/2	0/3	-	8
	1.0 *	0/5	0/2	0/2	0/3	-	7
1050°F	2 x 7	5/5	2/2	2/2	3/3	-	14
	7.0	3/5	2/2	2/2	1/3	-	13
	3.5	1/4	0/2	2/2	1/3	-	3
	1.0	3/5	1/2	1/2	2/3	-	10
	0.33	0/5	0/2	0/2	0/3	-	11
	3.5 *	3/5	1/2	1/2	0/3	-	9
	1.0 *	0/5	0/2	2/2	0/3	-	12

* Rate of liquid delivery was about one-half that in other experiments of similar duration.

Table 22

Incidence of Mortality in Relation to Species and to the Temperature of the Inconel Tube

Species	Number of Animals that Died/ Number of Animals Exposed		
	1250°F	1050°F	Both Temperatures
	Tricresyl Phosphate		
	%	%	%
Mice	17/35 (48.6)	15/34 (44.1)	32/69 (46.4)
Guinea Pigs	7/14 (50.0)	10/14 (71.4)	17/28 (60.7)
Rabbits	2/21 (9.5)	7/21 (33.3)	9/42 (21.4)
Rats	5/14 (35.7)	6/14 (42.9)	11/28 (39.3)
Total	31/84 (36.9)	38/83 (45.8)	69/167(41.3)

Table 23

Concentration of Various Decomposition Products in Air of Chamber in the Several Experiments

(Rate of Air Flow through Furnace; 20 l/min)

Range of Variation in Temperature of Inconel °F	Experiment Number	Rate of Delivery of Liquid ml/min	Expressed as mg/l Above and ppm Below		
			Tricresyl Phosphate	Free Cresols	Carbon Monoxide
1240 - 1258	15	0.144	-	0.0049 1.1	-
1249 - 1252	2	0.122	0.0013 0.9	0.00266 0.6	0.0292 26.4
1247 - 1253	1	0.121	0.0088 6.0	0.00158 0.4	0.0345 31.1
1257	4	0.233	-	0.00676 1.6	-
1249	6	0.210	-	-	-
1245	8	0.071	-	0.0011 0.3	-
1258	7	0.072	-	-	-
1045 - 1057	14	0.130	-	0.011 2.6	-
1049 - 1051	13	0.125	-	0.098 23	-
1025	3	0.120	0.550 37.8	0.0565 13	0.0250 22.6
1053	10	0.175	-	-	-
1051	11	0.195	-	-	-
1049 - 1051	9	0.088	-	0.35 81	-
1051	12	0.082	-	-	-

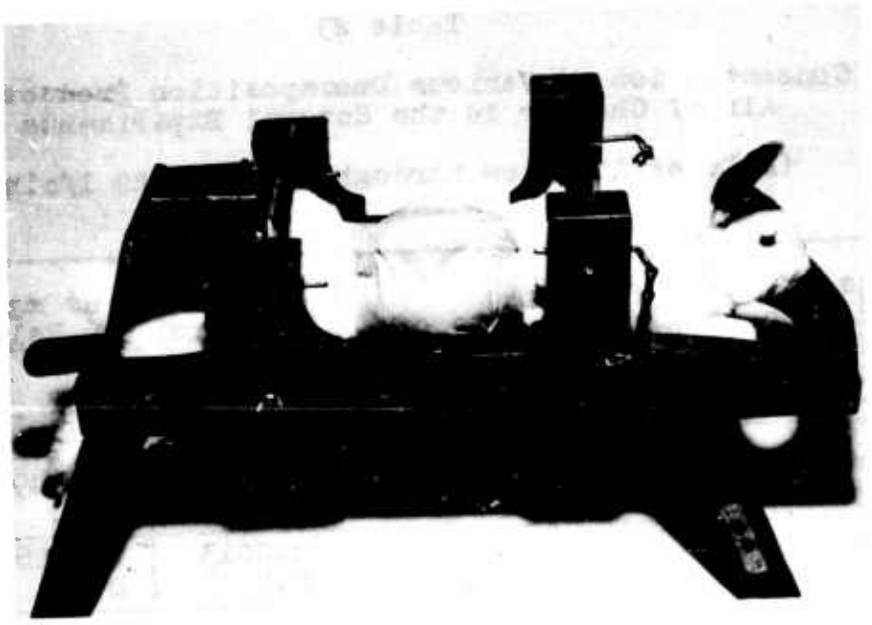

Figure 1. Stocks for Restraining Rabbits in Prone Position During Cutaneous Contacr Over a Period of Twenty-four Hours

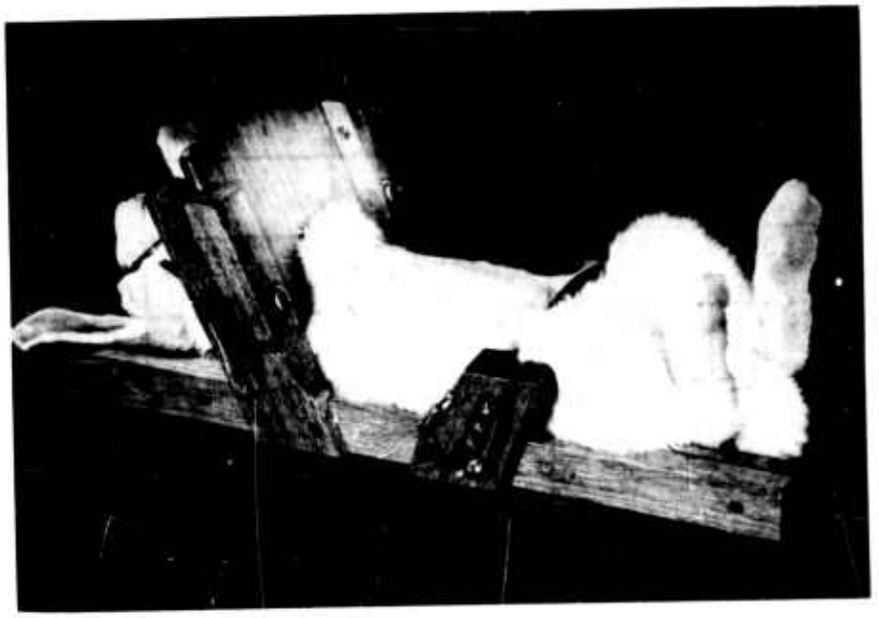

Figure 2. Stocks for Restraining Rabbits in Supine Position When Cutaneous Applications Were Made Repeatedly

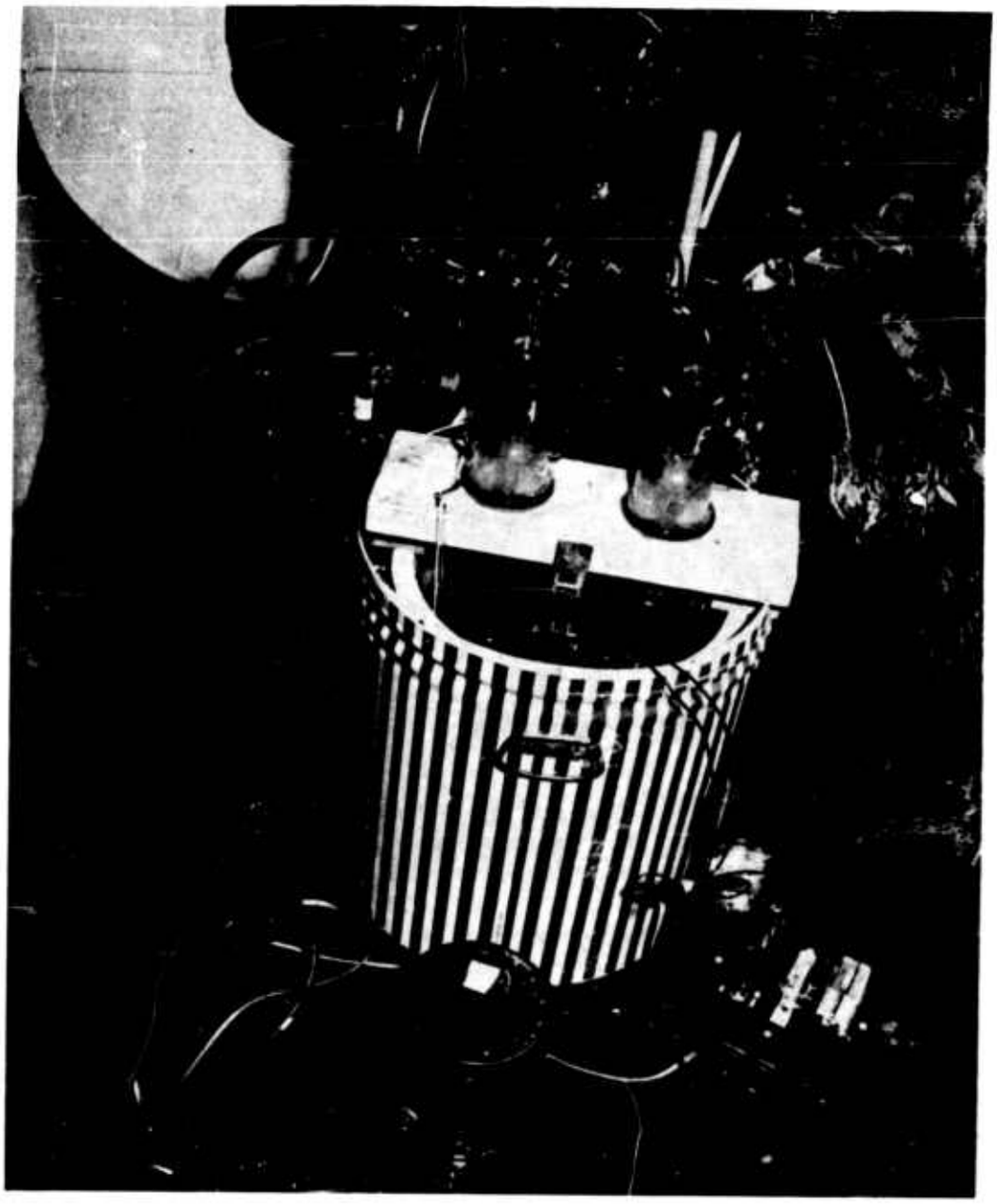

Figure 3. Towers from Which Tricresyl Phosphate was Volatilized as a Vapor

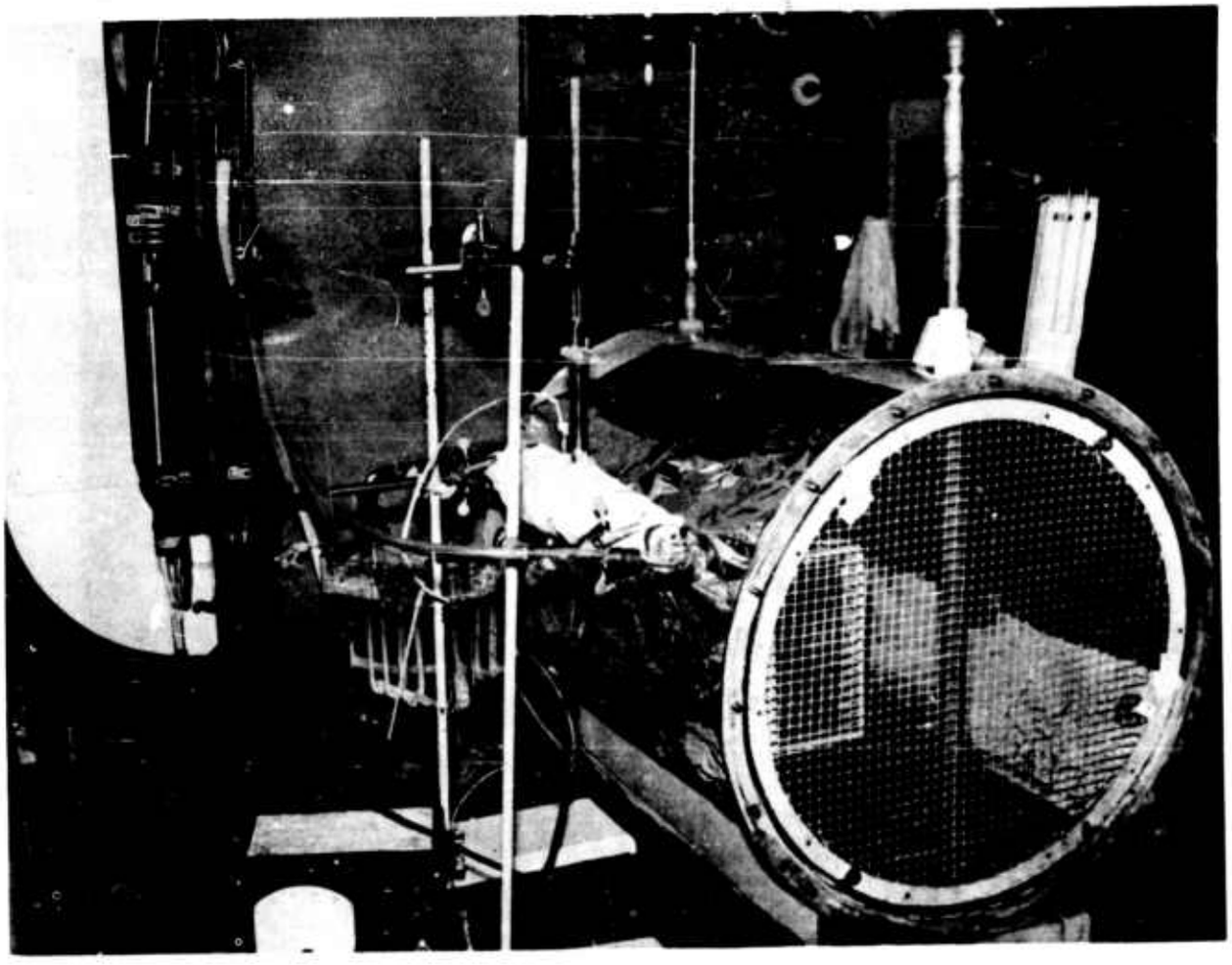

Figure 4. Equipment for the Generation of the Decomposition Products of Tricresyl Phosphate at High Temperatures

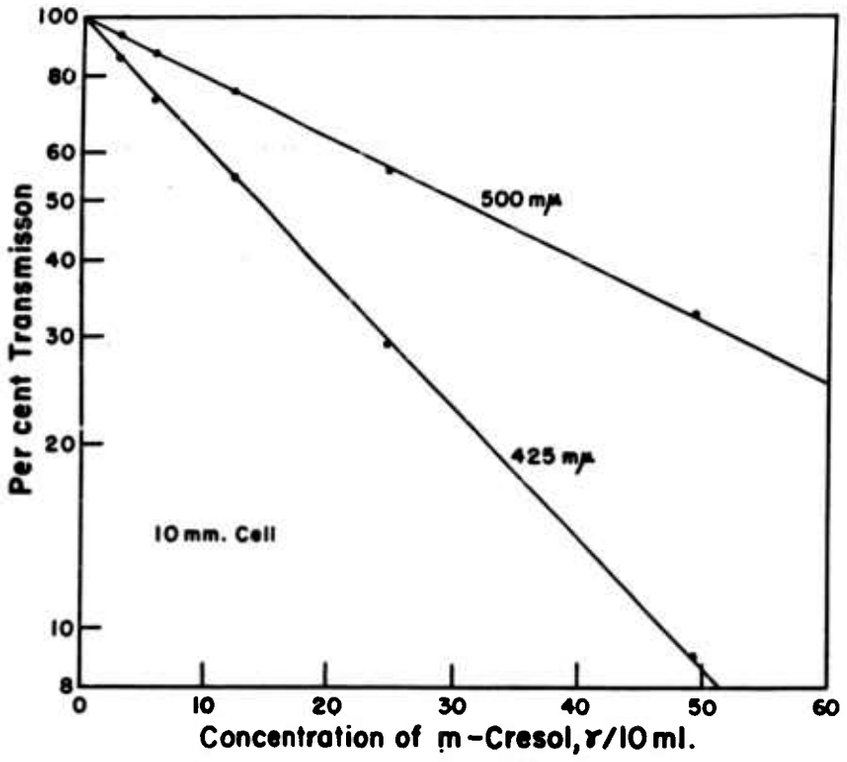

Figure 5. Standardization Curve for the Determination of Meta-Cresol

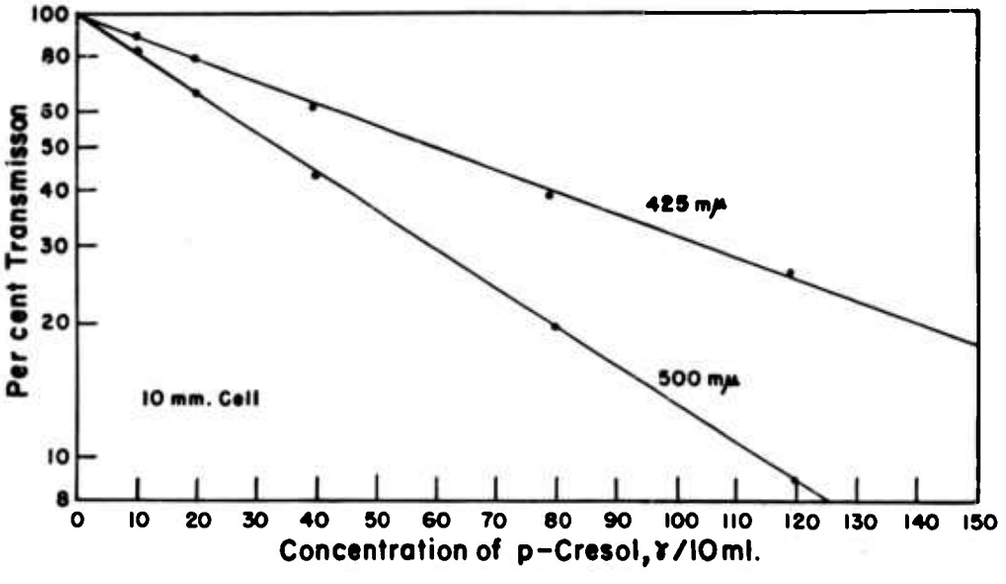

Figure 6. Standardization Curve for the Determination of Para-Cresol

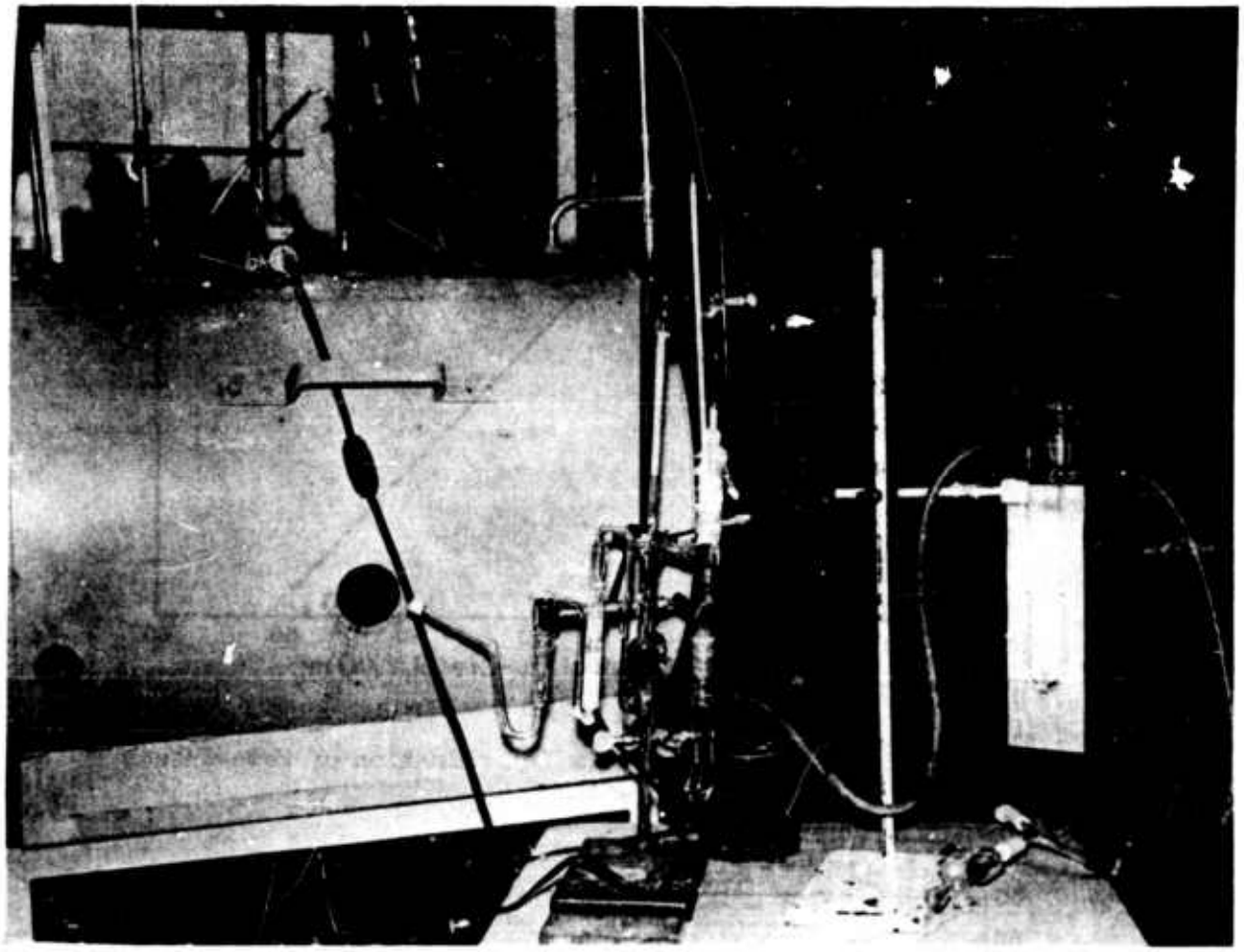

Figure 7. Absorption Train for the Collection of Carbon Monoxide from Air of Chamber

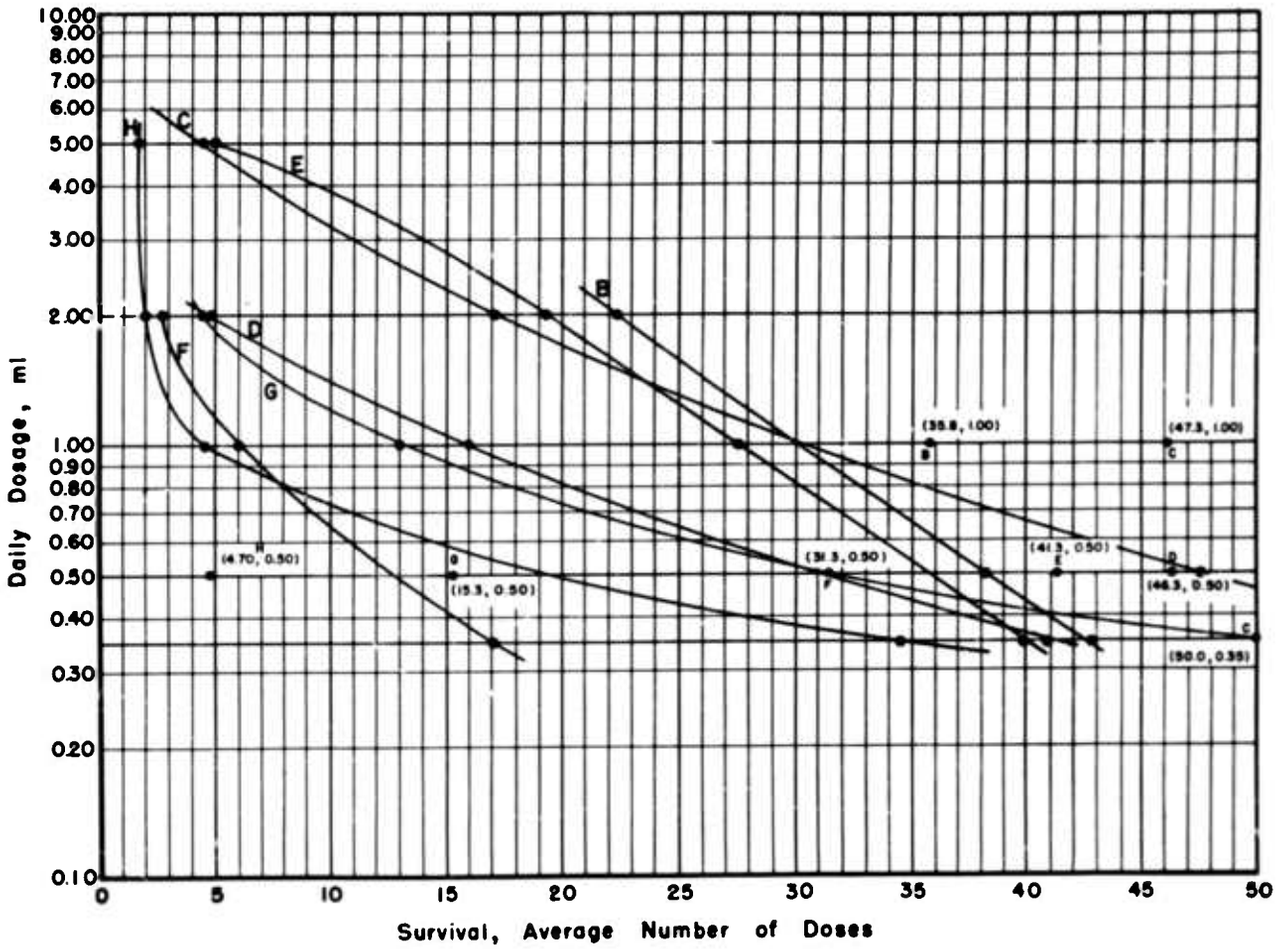

Figure 8. The Relationship Between the Length of Survival of Rabbits and the Dosage of Commercial Grades of Tricresyl Phosphate Maintained upon Their Skin for Two Hours Per Day on Five Days Per Week Over a Period of Several